AMPK alpha 2 (human; residues 352-366), pAb

Alternate Names: 5'-AMP-activated protein kinase catalytic subunit alpha-2, Acetyl-CoA carboxylase kinase, ACACA kinase, Hydroxymethylglutaryl-CoA reductase kinase, HMGCR kinase

Cat. No.	**68-0055-100**	Quantity:	100 µg
Lot. No.	**30295**	Storage:	-20˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS

This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (University of Dundee, Dundee, UK).

Background

Protein ubiquitylation and protein phosphorylation are the two major mechanisms that regulate the functions of proteins in eukaryotic cells. However, these different posttranslational modifications do not operate independently of one another, but are frequently interlinked to enable biological processes to be controlled in a more complex and sophisticated manner. Studying how protein phosphorylation events control the ubiquitin system and how ubiquitylation regulates protein phosphorylation has become a focal point of the study of cell regulation and human disease. Cloning of human 5'-AMP-activated protein kinase catalytic subunit alpha-2 (AMPK alpha 2) was first described by Stapleton *et al.* (1997). An example of such interplay between phosphorylation and ubiquitylation has been highlighted in recent studies indicating that AMPK alpha, along with AMPK kinases NUAK1 and MARK4, can be ubiquitylated with atypical ubiquitin chains. The deubiquitylating enzyme (DUB) found to remove these ubiquitin chains from both NUAK1 and MARK4 has been identified as USP9X (Zungu *et al.*, 2011). AMPK activation has also been shown to increase the expression of the E3 ubiquitin ligases MAFBx/Atrogin-1 and MuRF1. These ubiquitin ligases regulate key cardiac transcription factors to control cardiomyocyte mass and

Continued on page 2

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: AMPK alpha 2 (residues 352-366) [CMDDSAMHIPPGLKPH]

Purification: affinity-purified using immobilized immunogen

Formulation: phosphate-buffered saline

Specificity: detects AMPK alpha 2 at ~62 kDa

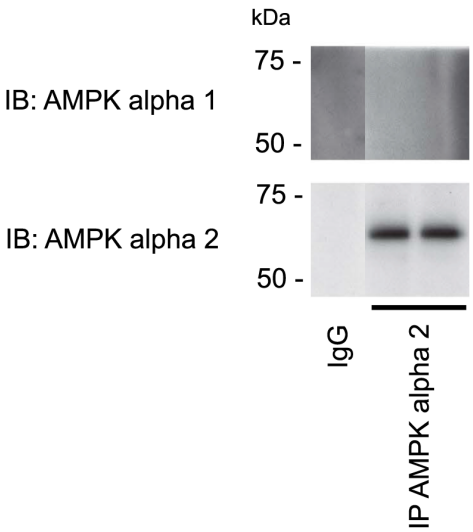
Reactivity: human; other species not tested

Stability/Storage: 12 months at -20˚C; aliquot as required

Research Applications and Quality Assurance

Western Immunoblotting:
use 1 µg/ml

Immunoprecipitation:
use 4 µg/mg of cell extract



Immunoprecipitation Assay:
Immunoprecipitation was performed from quad muscle lysate (1 mg) using 4 µg anti-AMPK alpha 2 antibody (Cat# 68-0055-100). The immunoprecipitates were subsequently analysed by Western Blot using a commercially available anti-AMPK alpha 1 antibody or anti-AMPK alpha 2 antibody (Cat# 68-0055-100). The images indicate that the anti-AMPK alpha 2 antibody (Cat# 68-0055-100) does not immunoprecipitate AMPK alpha 1.

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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CERTIFICATE OF ANALYSIS | **Page 2 of 2**

Background

Continued from page 1

remodeling, thus suggesting another mechanism by which AMPK may function in the heart. The relevance of AMPK ubiquitylation in cardiac disease has yet to be tested directly, but it likely represents an important mechanism that occurs in common cardiac diseases that may be targeted for therapy (Zungu *et al.*, 2011).

Antibody Production:

Anti-AMPK alpha 2 (human) polyclonal antibody was raised in sheep against AMPK alpha 2 (residues 352-366 of human AMPK alpha 2). The antibodies were purified by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (MRC-PPU, University of Dundee, Dundee, U.K.) by affinity purification of the anti-AMPK alpha 2 pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-AMPK alpha 2 (human) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Stapleton D, Woollatt E, Mitchelhill KI, Nicholl JK, Fernandez CS, Michell BJ, *et al.* (1997) AMP-activated protein kinase isoenzyme family: subunit structure and chromosomal location. *FEBS Lett* **409**, 452-456.

Zungu M, Schisler JC, Essop MF, McCudden C, Patterson C and Willis MS (2011) Regulation of AMPK by the ubiquitin proteasome system. *Am J Pathol* **178**, 4-11.

Application References:

Durante PE, Mustard KJ, Park SH, Winder WW and Hardie DG (2002) Effects of endurance training on activity and expression of AMP-activated protein kinase isoforms in rat muscles. American journal of physiology. *Endocrinology and Metabolism* **283** E178-186.

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Hawley SA, Gadalla AE, Olsen GS and Hardie DG (2002) The antidiabetic drug metformin activates the AMP-activated protein kinase cascade via an adenine nucleotide-independent mechanism. **Diabetes 51**, 2420-2425.

McGee SL, Mustard KJ, Hardie DG and Baar K (2008) Normal hypertrophy accompanied by phosphoryation and activation of AMP-activated protein kinase alpha1 following overload in LKB1 knockout mice. *J Physiol* **586**, 1731-1741.

Salt I, Celler JW, Hawley SA, Prescott A, Woods A, Carling D, *et al.* (1998) AMP-activated protein kinase: greater AMP dependence, and preferential nuclear localization, of complexes containing the alpha2 isoform. *Biochem J* **334** (Pt 1), 177-187.

Woods A, Salt I, Scott J, Hardie DG and Carling D (1996) The alpha1 and alpha2 isoforms of the AMP-activated protein kinase have similar activities in rat liver but exhibit differences in substrate specificity *in vitro*. *FEBS* Lett **397**, 347-351.

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